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Detection of the putative E2 protein of hepatitis C virus in human liver.

Nakamoto Y, Kaneko S, Honda M, Unoura M, Cheong J, Harada A, Matsushima K, Kobayashi K, Murakami S.

First Department of Internal Medicine, Faculty of Medicine, Kanazawa University, Ishikawa, Japan.

The question was asked whether a predicted envelope protein, considered to be processed from the polyprotein precursor encoded by the putative E2/NS1 region of the hepatitis C virus (HCV) genome, may be observed in HCV-infected humans. Two polyclonal antibodies against recombinant E2/NS1 proteins were prepared and their reactivity tested against liver extracts from HCV-infected patients by immunoblotting analysis. A band corresponding to a size of 44 kDa was detected in liver extracts from patients who were positive for the HCV-specific antibody anti-C100-3 but not in liver extracts from patients who did not have anti-C100-3 antibody. Additionally, no band was detected using preimmune sera or antisera which had been preabsorbed with recombinant E2/NS1 proteins. Deglycosylation studies demonstrated that the 44 kDa protein was a glycosylated form of a 38 kDa protein which corresponds to the predicted molecular weight of the putative E2/NS1 protein. These results suggest that the 44 kDa protein is a product of the E2/NS1 region. Frequent observation of the 44 kDa band in cases of chronic active hepatitis C suggests a correlation between the expression of this protein and the progression of hepatitis.

PMID: 7519251 [PubMed - indexed for MEDLINE]

1: Virology 1994 Apr;200(1):246-55 Related Articles, Links

Characterization and mapping of a B-cell immunogenic domain in hepatitis C virus E2 glycoprotein using a yeast peptide library.

Mink MA, Benichou S, Madaule P, Tiollais P, Prince AM, Inchauspe G.

Laboratory of Virology and Parasitology, Lindsley F. Kimball Research Institute of the New York Blood Center, New York 10021.

To identify conserved humoral antigenic determinants within the hepatitis C virus (HCV) envelope protein E2, we expressed a peptide library containing random short fragments of the HCV envelope in yeast. Clones were identified using a monospecific rabbit antibody to a region downstream of the E2 hypervariable region. The clones define the limits of two original antigenic domains: a major one (aa 493-576) and a minor one (aa 535-606). The major antigenic domain maps in a region that displays a high degree of homology within a (HCV) subtype (92-97.6% identity). Yeast-encoded determinants were characterized by Western blot analysis, N-glycosidase F digestion, and using a panel of synthetic peptides. The data suggest that the major antigenic domain contains at least two determinants, one of them mimicked by an 18-mer peptide (aa 514-531). ELISA and competitive inhibition assays demonstrated that: (1) the determinants appear subtype 1a-specific, (2) seroprevalence of antibody to the determinants is rather low (20.6%), and (3) donors show a heterologous response to the different determinants. Antibody response to the E2 determinants was studied in HCV-infected chimpanzees and post-transfusion-associated NANB hepatitis cases. The antibody response was found during chronic infection and may not be effective for virus clearance.

PMID: 7510436 [PubMed - indexed for MEDLINE]

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Hypervariable 5'-terminus of hepatitis C virus E2/NS1 encodes antigenically distinct variants.

Lesniewski RR, Boardway KM, Casey JM, Desai SM, Devare SG, Leung TK, Mushahwar IK.

Experimental Biology Research, Abbott Laboratories, North Chicago, IL 60064.

Synthetic peptides representing sequences encoded at the 5'-terminus of E2/NS1 in hepatitis C virus (HCV) were constructed. Peptides synthesized based on the sequences of four distinct HCV isolates were used to develop enzyme immunoassays (EIAs) for detection of antibodies in chronic HCV patients and in HCV-infected plasma donors. HCV sequence-specific antibodies were detected among patients with chronic HCV from the United States and Italy at frequencies of 22.2% and 55.8%, respectively. Similarly, sequence-specific antibodies were detected in 54.6% of U.S. and 55.6% of Japanese commercial plasma donors who had previous evidence of HCV exposure. Our data support earlier findings of geographic variability among HCV variants. The region encoded by amino acids (aa) 380-436 was shown to contain at least one variant-specific and one conserved epitope. The data further indicate that a majority of patients chronically infected with HCV (58.1% U.S., 68.8% Italy) have antibodies directed to the 5'-terminus of the E2/NS1 gene product. We conclude that genotypic variability within the E2/NS1 gene of HCV results in antigenically distinct variants.

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1: Hepatology 1993 May;17(5):763-71

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Characterization of hepatitis C virus structural proteins with a recombinant baculovirus expression system.

Hsu HH, Donets M, Greenberg HB, Feinstone SM.

Department of Medicine, Veterans Affairs Palo Alto Medical Center, California 94304.

We cloned and expressed the sequences encoding the structural proteins of the hepatitis C virus in a baculovirus eukaryotic expression system. Four recombinant constructs expressed sufficient hepatitis C virus-specific proteins in insect cell culture to allow analysis of protein cleavage, glycosylation and immunoreactivity. Using immunoblot analysis, we detected a 22-kD protein corresponding to the hepatitis C virus capsid protein cleaved from a larger precursor. Recombinant constructs encoding the presumptive envelope (E1) protein produced products ranging from 30 to 35 kD, whereas constructs encoding the presumptive E2/NS1 protein expressed products ranging in size from 68 to 73 kD. The recombinant envelope proteins were glycosylated, as shown by sensitivity to endoglycosidase F digestion, whereas the capsid was not. We examined the immunoreactivity of these recombinant proteins using sera from 50 patients chronically infected with HCV. Forty-seven of 50 of these sera contained antibodies against the capsid, 14 (28%) also had antibodies against E1 and at least 5 (10%) had antibody against E2/NS1. Forty-seven of 50 sera (94%) were viremic, as determined on hepatitis C virus polymerase chain reaction. The three sera that were hepatitis C virus polymerase chain reaction negative did not have envelope antibodies, whereas all sera that had envelope antibodies were also hepatitis C virus polymerase chain reaction positive. Thus antibodies to baculovirus-expressed hepatitis C virus structural proteins, including E1 and E2/NS1, are found in the presence of viremia.

PMID: 8387945 [PubMed - indexed for MEDLINE]

1: Gut 1993;34(2 Suppl):S64-5 Related Articles, Links

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Expression of HCV E2/NS1 protein as a fusion protein with maltose binding protein: detection of anti-E2/NS1 antibody in chronic liver disease.

Yokosuka O, Omata M, Ito Y, Ohto M.

First Department of Medicine, Chiba University School of Medicine, Japan.

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The presence of anti-E2 antibody was investigated in the serum samples of 46 patients with liver disease, who were positive for hepatitis C virus-RNA, and in five subjects HCV-RNA-negative acting as controls. Antibody to E2/NS1 protein was found in seven of 46 (15%) of the patients with liver disease but in none of the control subjects. In one patient who was treated successfully with interferon, the levels of anti-E2 gradually decreased and then finally disappeared after treatment. This suggests that the E2/NS1 protein may play a role in active viral replication.

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Expression and characterization of glycoprotein gp35 of hepatitis C virus using recombinant vaccinia virus.

Kohara M, Tsukiyama-Kohara K, Maki N, Asano K, Yamaguchi K, Miki K, Tanaka S, Hattori N, Matsuura Y, Saito I, et al.

Fundamental Research Laboratory, Tonen Co. Nishi-Tsurugaoka, Saitama, Japan.

Complementary DNA clones corresponding to one of the putative structural regions of the hepatitis C virus (HCV) genome were obtained from sera of non-A non-B hepatitis patients. The putative envelope gene was expressed by using a recombinant vaccinia virus carrying this region of the HCV genome. In cells infected with the recombinant vaccinia virus, a glycosylated protein with an M(r) of about 35K (gp35) was specifically detected by convalescent sera from hepatitis C patients. The sera from rabbits immunized with this recombinant vaccinia virus reacted to the gp35 produced in insect cells and also to gp35 which was translated in vitro in the glycosylated and processed form. The gp35 was used to detect antibodies in sera of only 7 to 23% of HCV patients at various stages of HCV disease. These results suggest that the gp35 of HCV may not have high antigenicity in humans.

PMID: 1328487 [PubMed - indexed for MEDLINE]

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Expression of MBP-HCV NS1/E2 fusion protein in E. coli and detection of anti-NS1/E2 antibody in type C chronic liver disease.

Mita E, Hayashi N, Ueda K, Kasahara A, Fusamoto H, Takamizawa A, Matsubara K, Okayama H, Kamada T.

First Department of Medicine, Osaka University Medical School, Japan.

To characterize the putative NS1/E2 (non-structural protein 1/envelope 2) domain of HCV (hepatitis C virus), we expressed the hydrophilic three-quarters of this domain in a form of MBP (maltose binding protein) fusions in Escherichia coli. When we checked the positive frequency of antibody to this fusion protein, 17% of patients with type C chronic liver disease had this antibody. However, they were all positive for HCV-RNA in sera. These results suggest that the appearance of anti-NS1/E2 antibody does not serve as evidence of viral clearance.

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Establishment of a cell line constitutively expressing E2 glycoprotein of hepatitis C virus and humoral response of hepatitis C patients to the expressed protein.

Harada S, Suzuki R, Ando A, Watanabe Y, Yagi S, Miyamura T, Saito I.

Department of Medical Entomology, National Institute of Health, Tokyo, Japan.

A Chinese hamster ovary cell line was established which abundantly expresses the second envelope protein (E2) of hepatitis C virus under the control of an exogenous promoter. The expressed E2 protein was found to be a glycoprotein of 58 kDa by immunoprecipitation with sera from patients that had chronic hepatitis C. Using this cell line as antigen in immunofluorescence tests, as high as 93% of patients with non-A non-B hepatitis had antibodies against E2 protein. In Western blots using SDS-denatured E2 protein, however, the detectability of the antibody was drastically reduced to 30%. Immunoprecipitation assays and ELISA, using both native and denatured E2 protein, revealed that antibodies to E2 protein were present in most of the chronic hepatitis C patients and that they reacted only to the native forms.

PMID: 7730806 [PubMed - indexed for MEDLINE]

1: J Viral Hepat 1995;2(5):227-34 Related Articles, Links

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Identification of hepatitis C virus by immunoelectron microscopy.

Li X, Jeffers LJ, Shao L, Reddy KR, de Medina M, Scheffel J, Moore B, Schiff ER.

Center for Liver Diseases, University of Miami School of Medicine, Veterans Administration Medical Center, FL 33125 USA.

Sequencing of the hepatitis C virus (HCV) has provided a better understanding of the natural history, immunology, and epidemiology of this virus. However, the morphology of HCV has not been definitively characterized. In this study, through a sequence of concentration processes, virus-like particles were isolated from human serum and liver tissue, visualized by transmission electron microscopy and identified as hepatitis C virion by immunoelectron microscopy. Spherical flavi-like virus particles, approximately 70 nm in diameter, were observed in the fraction with 1.04-1.12 g ml-1 sucrose density and bound to immunogold particles with monoclonal antibodies (mAb) against hepatitis C. The nucleocapsid of the particles, which were 50 nm in diameter, appeared to be icosahedral in structure and surrounded by an envelope covered with surface projections. A 'tadpole' form of particles was also observed. The findings indicate that the low buoyant density in sucrose and the morphological features of the hepatitis C virion are consistent with the characteristics of flaviviruses and pestiviruses.

PMID: 8745314 [PubMed - indexed for MEDLINE]

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